AMMONIUM PERCHLORATE: EFFECT ON IMMUNE FUNCTION

QUALITY ASSURANCE AUDIT

Study No.: BRT 19990524 – Plaque-Forming Cell (PFC) Assay
Study No.: BRT 19990525 – Local Lymph Node Assay (LLNA) in Mice

Study Sponsor:	PSG Michael Girard
Study Monitor:	Michael L. Dourson, PhD, DABT Joan Dollarhide TERA 1757 Chase Avenue Cincinnati, OH 45223
Study Performed At:	BRT-Burleson Research Technologies, Inc. 5706 Chapel Hill Road Raleigh, NC 27607
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Report Date:	June 30, 2000

The audit revealed that data calculations were performed as described in the protocol. Furthermore, all data

This portion of the audit included:

- Comparison of data contained in the manually-recorded study records against the data shown in the computer generated spreadsheets, including cell viability and PFC values for duplicates at two dilutions (one mouse/group, 10% audit);
- Comparison of computer spreadsheet values against reported values for PFC/10^3 spleen cells, PFC/10^6 spleen cells, and SE, and SI (100% audit).

The audit revealed that data calculations were performed as described in the protocol. Furthermore, the data shown in the report graphs and tables were accurate and could be directly traced to the study records.

Consistency Between Protocol Requirements and Study Records

For this portion of the audit, I reviewed study records for the initial 90-day LLNA study. The records reviewed included:

- Weekly dosing calculations;
- Weekly actual dose calculation values documented in computer-generated spreadsheets;
- Weekly actual dose calculation values calculated manually for selected cages;
- Daily water weights;
- Weekly individual mouse weights;
- Weekly preparation of AP;
- Preparation and injection of cyclophosphamide;
- Preparation of vehicle and 0.25% DNCB;
- Administration of DNCB and subsequent harvest of lymph nodes.

The study records listed above documented that:

- Mice were housed five per cage;
- Each treatment and control group contained 10 animals;
- Compound was administered through water intake, and water bottles were weighed daily to determine intake;
- Dosing began on July 13, 1999 and ended on October 18, 1999;
- Doses were adjusted once per week based on the mean mouse body weight per cage;
- Calculations of actual doses received (mg/kg/day) were performed once per week;
- Actual dose calculation values in the computer-generated spreadsheets were consistent with the actual dose calculation values calculated manually (audit of one cage per week);
- Ammonium perchlorate stock solutions were prepared once per week;
- Cyclophosphamide was injected i.p. daily for five days prior to lymph node harvest, and the dose was calculated based on individual mouse weights (15 mg/kg);

- A 0.25% solution of DNCB in acetone/olive oil (4:1) was prepared fresh daily and administered daily for three days, followed by a two-day rest period and subsequent lymph node harvest;
- Lymph nodes were harvested approximately 5 hours after the first injection of ^{125}IUDR (2 uCi);
- Cell suspensions, four background vials, and a 1:500 dilution of the 8 uCi/mL ^{125}IUDR stock concentration were counted in the gamma counter.

Overall, the findings noted above were consistent with study protocol requirements. Deviations from the protocol are described below.

Deviations from the Protocol

The following deviations from the study protocol were noted:

- The doses of AP used during the initial 14- and 90-day portions of the study were 5-fold lower than those required by the protocol. As a result, the Sponsor verbally requested that these portions of the study be repeated using a 50 mg/kg/day dose.
- Animals in the initial and high-dose 90-day portions of the LLNA study were dosed with AP for 97 days rather than 90 days.
- Control animals in the initial and high-dose 90-day portions of the LLNA study received cyclophosphamide on Day 92, rather than on Day 85 as specified in the protocol.
- Changes to the protocol were approved verbally, as allowed by the protocol. However, the dates and the reasons for the changes were not subsequently put into writing and signed by the Study Director and the Sponsor's Divisional Toxicologist, as required by the protocol.
- For the LLNA study, the 4 background samples were placed at the beginning of each sample run, rather than being split between the beginning and the end of the run as stated in the protocol.

6/30/00